```
=> PreS1 (l) HBV
L1          262 PRES1 (L) HBV

=> Fusion (w) protien
L2           13 FUSION (W) PROTIEN

=> L1 and L2
L3            0 L1 AND L2

=> "tetanus toxin"
L4         4062 "TETANUS TOXIN"

=> L4 and L1
L5            1 L4 AND L1

=> "fusion protein"
L6        44346 "FUSION PROTEIN"

=> L6 and L1
L7           43 L6 AND L1

=> L4 and L7
L8            1 L4 AND L7

=> D L8 IBIB TI SO AU ABS
```

L6 ANSWER 5 OF 44346 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:731045 CAPLUS
TITLE: A nucleic acid construct encoding a processing component derived from then-terminal region of the hepatitis virus orf2, and an antigenic polypeptide
INVENTOR(S): Li, Fan; Anderson, David Andrew; Purcell, Damian Francis John
PATENT ASSIGNEE(S): MacFarlane Burnet Centre for Medical Research Limited,
Australia
SOURCE: PCT Int. Appl., 47 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001073078	A1	20011004	WO 2001-AU353	20010330

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: AU 2000-6616 A 20000331
TI A nucleic acid construct encoding a processing component derived from then-terminal region of the hepatitis virus orf2, and an antigenic polypeptide
SO PCT Int. Appl., 47 pp.
CODEN: PIXXD2
IN Li, Fan; Anderson, David Andrew; Purcell, Damian Francis John
AB A method for enhancing an immune response to a nucleic acid vaccine comprising administering to an animal a nucleic acid construct encoding a **fusion protein** comprising a processing component and an antigenic polypeptide of interest wherein said processing component provides heterogeneous processing of the antigenic polypeptide when the nucleic acid construct is expressed in a host cell and a resulting enhancement of the immune response. The processing component is derived from an N-terminal portion of PORF2 of Hepatitis E virus.

=> D L5 IBIB TI SO AU ABS

L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:223054 CAPLUS
DOCUMENT NUMBER: 130:266359
TITLE: Hepatitis B virus fusion polypeptides (**tetanus toxin** fused to pre-S1 antigen and/or pre-S2 antigen) and their use in the prevention or treatment of HBV infections
INVENTOR(S): Chatfield, Steven Neville
PATENT ASSIGNEE(S): Medeva Europe Limited, UK
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9915671	A1	19990401	WO 1998-GB2852	19980921
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9891744	A1	19990412	AU 1998-91744	19980921
EP 1015593	A1	20000705	EP 1998-944071	19980921
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001517447	T2	20011009	JP 2000-512962	19980921
NO 2000001397	A	20000505	NO 2000-1397	20000317

PRIORITY APPLN. INFO.: GB 1997-20033 A 19970919
WO 1998-GB2852 W 19980921

TI Hepatitis B virus fusion polypeptides (**tetanus toxin** fused to pre-S1 antigen and/or pre-S2 antigen) and their use in the prevention or treatment of HBV infections

SO PCT Int. Appl., 30 pp.
CODEN: PIXXD2

IN Chatfield, Steven Neville

AB The present invention provides polypeptides comprising **tetanus toxin** fragment C, or a fragment thereof, fused to the pre-S1 region of hepatitis B virus (HBV), or a fragment thereof, and/or the pre-S2 region of HBV or a fragment thereof. The present invention also provides polynucleotides encoding the fusion polypeptides of the invention. The invention further provides vectors comprising a polynucleotide encoding a polypeptide of the invention operably linked to the promoter region of gene htrA and a host cell transfected with these vectors. The polypeptides, polynucleotides, and vectors may be used in the prevention or treatment of HBV infections. Still further, the invention provides a vaccine compn. comprising a polypeptide, polynucleotide or vector of the invention together with a pharmaceutically acceptable carrier diluent. Finally, the invention produces a method for producing antibodies which recognize epitopes within the pre-S1 and/or pre-S2 regions of HBV and use of these antibodies in treatment of HBV infections.

REFERENCE COUNT: 8
REFERENCE(S):
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(2) Khan, C; PNAS, U S A 1994, V91(23), P11261 CAPLUS
(3) Medeva Holdings BV; WO 9403615 A 1994 CAPLUS
(4) Medeva Holdings BV; WO 9504151 A 1995 CAPLUS
(5) Medeva Holdings BV; WO 9520665 A 1995 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> PreS (l) HBV
L9 228 PRES (L) HBV

=> L9 (l) L4
L10 0 L9 (L) L4

=> L9 and L4
L11 0 L9 AND L4

=> L9 and L6
L12 18 L9 AND L6

=> D L12 IBIB TI SO AU ABS 1-18

L12 ANSWER 1 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:430420 CAPLUS
DOCUMENT NUMBER: 134:191934
TITLE: IL-2 and **HBV preS fusion protein** expression plasmid transfer into mouse muscle by jet-gun in vivo
AUTHOR(S): Shen, Xiaofang; Ma, Dalong; Bai, Huiqing; Li, Jianyuan; Chen, Zhangguo
CORPORATE SOURCE: Department of Immunology, Beijing Medical University, Beijing, 100083, Peop. Rep. China
SOURCE: Zhongguo Mianyixue Zazhi (2000), 16(5), 241-243
CODEN: ZMZAEE; ISSN: 1000-484X
PUBLISHER: Zhongguo Mianyixue Zazhi Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
TI IL-2 and **HBV preS fusion protein** expression plasmid transfer into mouse muscle by jet-gun in vivo
SO Zhongguo Mianyixue Zazhi (2000), 16(5), 241-243
CODEN: ZMZAEE; ISSN: 1000-484X
AU Shen, Xiaofang; Ma, Dalong; Bai, Huiqing; Li, Jianyuan; Chen, Zhangguo
AB The efficiency of DNA immunization by jet-gun i.m. injection of eukaryotic expression plasmid pCWIIP, which expresses the **fusion protein**, IL-2-preS, was studied. Balb/c mice were divided into 2 groups: jet-gun and syringe. Each animal was immunized with pCWIIP, pCIL-2 and pCI, resp. Local muscles were obtained, and IL-2-preS expression were detected immunohistochem. 4 days later. PCWIIP, pCIIL-2 and pCI were injected into Balb/c mice via jet-gun, syringe, and epidermal. The blood samples were harvested from eyes of the treated mice at interval of 0, 2, 4, 6, 8 w, and their serum anti-preS IgG were measured by indirect ELISA. Immunohistochem. showed that the use of the jet-gun induced a significant higher expression in skeletal muscle cell than the use of syringe. Indirect ELISA showed that the levels of anti-preS IgG were in the following order from high to low: jet gun, i.m. injection, and epidermal groups. The jet-gun is a better methods than those of syringe and epidermal methods for the immunization with pCWIIP plasmid, and would make immunization faster and therefore less costly and dangerous.

L12 ANSWER 2 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:679409 CAPLUS
DOCUMENT NUMBER: 132:179413
TITLE: Immunogenicity of hepatitis B virus preS antigen and interleukin - 2 fused protein expressed by Cos - 7 cell

AUTHOR(S): Zhou, Weihong; Wan, Yanping; Chen, Zhangguo; Ma, Dalong
CORPORATE SOURCE: Department of pathogenetic laboratory technology, Hengyang medical college, Hengyang, 421001, Peop. Rep. China
SOURCE: Hengyang Yixueyuan Xuebao (1999), 27(3), 253-255
CODEN: HEYXES; ISSN: 1000-2510
PUBLISHER: Hengyang Yixueyuan Xuebao Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

TI Immunogenicity of hepatitis B virus preS antigen and interleukin - 2 fused protein expressed by Cos - 7 cell

SO Hengyang Yixueyuan Xuebao (1999), 27(3), 253-255
CODEN: HEYXES; ISSN: 1000-2510

AU Zhou, Weihong; Wan, Yanping; Chen, Zhangguo; Ma, Dalong

AB In order to combine the biofunctions of interleukin-2 (IL-2) and **preS** antigen (preSAg, **preS**) of hepatitis B virus (**HBV**) and search for the therapeutic agents specific for **HBV** persistent infection, we constructed the eukaryotic expression plasmid pCWIIP for IL-2preS, which could be secreted from the Cos-7 cells transfected with pCWIIP. The efficiency for Cos-7 cells to secretively express IL-2preS fused protein was identical to that to express IL - 2 and **preS** alone, but the fused protein might enhance the immunogenicity of the **preS** and improve immune response in human. The result laid an evidence of theory and expt. for the designation and construction of preventive and therapeutic drugs specific against **HBV** persistent infection.

L12 ANSWER 3 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:451839 CAPLUS
DOCUMENT NUMBER: 131:241687
TITLE: Expression and characterization of chimeric hepatitis B surface antigen particles carrying preS epitopes
AUTHOR(S): Hui, Jingyi; Li, Guangdi; Kong, Yuying; Wang, Yuan
CORPORATE SOURCE: Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai, Peop. Rep. China
SOURCE: J. Biotechnol. (1999), 72(1,2), 49-59
CODEN: JBITD4; ISSN: 0168-1656
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Expression and characterization of chimeric hepatitis B surface antigen particles carrying preS epitopes

SO J. Biotechnol. (1999), 72(1,2), 49-59
CODEN: JBITD4; ISSN: 0168-1656

AU Hui, Jingyi; Li, Guangdi; Kong, Yuying; Wang, Yuan

AB Many studies have provided evidence that hepatitis B surface antigen (HBsAg) including preS1 and preS2 sequences could be an ideal candidate for a new hepatitis B virus (**HBV**) vaccine with higher efficacy. However, the large (L) protein contg. the entire **preS** region expressed in mammalian cells is not efficiently assembled into particles and secreted. Here the authors report an alternative approach to include the dominant epitopes of preS1 and preS2 to the small (S) protein as **fusion proteins** by the recombinant DNA technol. Three **fusion proteins** contg. preS2(120-146) and preS1(21-47) at the N-terminus and/or truncated C-terminus of S protein were expressed

using the recombinant vaccinia virus system. All these **fusion proteins** were efficiently secreted in the particulate form, and displayed S, preS1 and/or preS2 antigenicity. Further anal. showed that these chimeric HBsAg particles elicited strong antibody responses against S, preS1 and preS2 antigens in BALB/c mice, suggesting that they could be promising candidates for a new recombinant vaccine to induce broader antibody response required for protection against hepatitis B viral infection.

REFERENCE COUNT: 37
REFERENCE(S): (2) Budkowska, A; Hepatology 1986, V6, P360 CAPLUS
(3) Cheng, K; J Virol 1986, V60, P337 CAPLUS
(5) Delpeyroux, F; Science 1986, V233, P472 CAPLUS
(6) Feng, Z; Acta Biochim Biophys Sin (in Chinese) 1987, V19, P428 CAPLUS
(8) Hui, J; Science in China (Series C) 1998, V41, P56 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 4 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:238067 CAPLUS
DOCUMENT NUMBER: 131:83621
TITLE: Constructing eukaryotic expression vector for hepatitis C virus E2 protein fused with hepatitis B virus preS protein and expressing fused protein in mammalian cells
AUTHOR(S): Xie, Yao; Tao, Qimin
CORPORATE SOURCE: Institute of Hepatology, People's Hospital, Beijing Medical University, Beijing, 100044, Peop. Rep. China
SOURCE: Beijing Yike Daxue Xuebao (1999), 31(1), 38-40
CODEN: BYDXEV; ISSN: 1000-1530
PUBLISHER: Beijing Yike Daxue
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
TI Constructing eukaryotic expression vector for hepatitis C virus E2 protein
fused with hepatitis B virus preS protein and expressing fused protein in mammalian cells
SO Beijing Yike Daxue Xuebao (1999), 31(1), 38-40
CODEN: BYDXEV; ISSN: 1000-1530
AU Xie, Yao; Tao, Qimin
AB The eukaryotic vector was constructed which expresses the combined protein
of hepatitis C virus (HCV) E2 and hepatitis B virus (**HBV**) **preS** proteins. HCV E2 and **HBV preS** genes were amplified with PCR and cloned into mammalian expression vector pcDNA3. The constructed vector was transfected into COS7 cells with lipofectin. The expressed E2-**preS** protein was detected using immunofluorescence. The chimeric gene, which was about 1.6 kb, included entire **HBV preS** and HCV E2 gene. The cells transfected with the constructed vector expressed E2-**pres** protein successfully. The constructed vector contg. chimeric gene of E2-**preS** protein expressed E2-**preS** protein in mammalian COS7 cells.

L12 ANSWER 5 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:430146 CAPLUS
DOCUMENT NUMBER: 127:148135
TITLE: The fusion expression of **HBV preS** epitopes and the core antigen

AUTHOR(S): Li, Yingchun; Xu, Xie; Chen, Zuoyi; Wang, Yuan; Li, Guangdi
CORPORATE SOURCE: Shanghai Institute Biochemistry, Academia Sinica, Shanghai, 200031, Peop. Rep. China
SOURCE: Shengwu Huaxue Yu Shengwu Wuli Xuebao (1996), 28(4), 380-388
CODEN: SHWPAU; ISSN: 0582-9879
PUBLISHER: Shanghai Kexue Jishu Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

TI The fusion expression of **HBV preS** epitopes and the core antigen
SO Shengwu Huaxue Yu Shengwu Wuli Xuebao (1996), 28(4), 380-388
CODEN: SHWPAU; ISSN: 0582-9879
AU Li, Yingchun; Xu, Xie; Chen, Zuoyi; Wang, Yuan; Li, Guangdi
AB The DNA fragments encoding the **preS** epitopes of hepatitis B virus (**HBV**) surface antigen were fused to the HBc gene and expressed in E. coli under the control of the tac promoter. The products were analyzed by ELISA and Western Blotting, which confirmed that the hybrid proteins were expressed as expected. Anal. by electron microscopy and CsCl d. gradient ultracentrifugation showed that all **fusion proteins** were able to form particles, only with a slightly lower d. than the native multimeric HBc. Partially purified fusion particles were then used as immunogen to Balb/c mice and higher titer antibody against the preS1 (21-47) epitope was obsd., which demonstrated that the immunogenicity of preS1 could be greatly improved when fused with the el loop in HBc protein.

L12 ANSWER 6 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:706179 CAPLUS
DOCUMENT NUMBER: 125:325783
TITLE: Specific binding of the hepatitis B virus preS antigen
to an EBV-transformed B-cell line
AUTHOR(S): Choi, Eun-A.; Park, Jung-Hyun; Cho, Eun-Wie; Hahm, Kyung-Soo; Kim, Kil Lyong
CORPORATE SOURCE: Peptide Engineering Research Unit, Korea Research Institute Bioscience Biotechnology, Taejon, 305-600, S. Korea
SOURCE: Mol. Cells (1996), 6(5), 622-627
CODEN: MOCEEK; ISSN: 1016-8478
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Specific binding of the hepatitis B virus preS antigen to an EBV-transformed B-cell line
SO Mol. Cells (1996), 6(5), 622-627
CODEN: MOCEEK; ISSN: 1016-8478
AU Choi, Eun-A.; Park, Jung-Hyun; Cho, Eun-Wie; Hahm, Kyung-Soo; Kim, Kil Lyong
AB Specific attachment onto the target cell is one of the mechanisms that causes the restricted and specific host cell range of viral pathogens. In the case of hepatitis B virus (**HBV**), human hepatocytes are known to be the major host cells onto which **HBV** is believed to bind by the **preS** region of its surface antigen (HBsAg). To examine the host cell range of **HBV**, cells have to be analyzed primarily upon their ability to bind the **preS** antigen. To do this, in this study, the **preS** region of **HBV** was expressed as a maltose binding protein (MBP) **fusion protein** in prokaryotes and used for detection of putative **HBV** receptors on

various cell lines. Among the cell lines investigated, we could identify one EBV-transformed B-cell line, Wa-cells, which showed specific binding to the MBP-**preS fusion protein** as revealed by FACS anal. The expression level of the **preS** binding protein on Wa-cells was comparable to that of cells of hepatic origin such as HepG2 cells. With the identification of a non-hepatic cell line that expresses putative **HBV** receptors, a novel way is opened for anal. of the host cell specificity of **HBV** as well as the possible pathogenicity of **HBV** in extra-hepatic tissues. Attempts for in vitro transfection of Wa-cells with **HBV** as well as the identification of **preS** binding proteins of these cells are under progress.

L12 ANSWER 7 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:477739 CAPLUS
DOCUMENT NUMBER: 125:189732
TITLE: Visualization of hepatitis B virus (HBV) surface protein binding to HepG2 cells
AUTHOR(S): Lee, Dong-Gun; Park, Jung-Hyun; Choi, Eun-A.; Han, Mi-Young; Kim, Kil-Lyong; Hahm, Kyung-Soo
CORPORATE SOURCE: Peptide Eng. Res. Unit, Korea Res. Inst. Biosci. Biotechnology, Taejon, 305-600, S. Korea
SOURCE: J. Biochem. Mol. Biol. (1996), 29(2), 175-179
CODEN: JBMBE5; ISSN: 1225-8687
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Visualization of hepatitis B virus (HBV) surface protein binding to HepG2 cells
SO J. Biochem. Mol. Biol. (1996), 29(2), 175-179
CODEN: JBMBE5; ISSN: 1225-8687
AU Lee, Dong-Gun; Park, Jung-Hyun; Choi, Eun-A.; Han, Mi-Young; Kim, Kil-Lyong; Hahm, Kyung-Soo
AB Viral surface proteins are known to play an essential role in attachment of the virus particle to the host cell membrane. In the case of hepatitis B virus (**HBV**), several reports described potential receptors on the target cell side but no definite receptor protein has been isolated yet. As for the viral side, it has been suggested that the **preS** region of the envelope protein, esp. the preS1 region, is involved in binding of **HBV** to the host cell. In this study, the preS1 region was recombinantly expressed in the form of a maltose-binding protein (MBP) **fusion protein** and used to identify and visualize the expression of putative **HBV** receptor(s) on the host cell. By using laser scanning confocal microscopy and FACS anal., MBP-preS1 proteins were shown to bind to the human hepatoma cell line HepG2 in a receptor-ligand specific manner. The binding kinetics of MBP-preS1 to its cellular receptor were temp. and time dependent. In cells permeabilized with Triton X-100 and treated with the **fusion protein**, a specific staining of the nuclear membrane could be obsd. To det. the precise location of the receptor binding site within the preS1 region, several short overlapping peptides from this region were synthesized and used in a competition assay. In this way, the receptor binding epitope in preS1 was revealed to be amino acid residues 27-51, which is in agreement with previous reports. These results confirm the significance of the preS1 region in virus attachment in general and suggest an internalization pathway mediated by direct attachment of the viral particle to the target cell membrane.

L12 ANSWER 8 OF 18 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:941482 CAPLUS
DOCUMENT NUMBER: 123:336912
TITLE: Fine mapping and functional characterization of two immuno-dominant regions from the preS2 sequence of hepatitis B virus
AUTHOR(S): Meisel, Helga; Sominskaya, Irina; Pumpens, Pauls; Pushko, Peter; Borisova, Galina; Deepen, Ralf; Lu, Xuangyong; Spiller, Gerald H.; Krueger, Detlev H.; et al.
CORPORATE SOURCE: Institute Medical Virology, Humboldt University, Berlin, D-35392, Germany
SOURCE: Intervirology (1995), Volume Date 1994, 37(6), 330-9
CODEN: IVRYAK; ISSN: 0300-5526
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Fine mapping and functional characterization of two immuno-dominant regions from the preS2 sequence of hepatitis B virus
SO Intervirology (1995), Volume Date 1994, 37(6), 330-9
CODEN: IVRYAK; ISSN: 0300-5526
AU Meisel, Helga; Sominskaya, Irina; Pumpens, Pauls; Pushko, Peter; Borisova, Galina; Deepen, Ralf; Lu, Xuangyong; Spiller, Gerald H.; Krueger, Detlev H.; et al.
AB A set of monoclonal antibodies (mAbs) directed against the preS2 region of hepatitis B virus (**HBV**) surface antigen (HBsAg) was generated by immunization of mice with native HBsAg isolated from the blood of **HBV** carriers. According to (1) mutual competition binding of mAb to natural HBsAg, (2) recognition of full-length preS2 displayed on hepatitis B core particles, (3) recognition of synthetic partial preS2 peptides, and (4) Western blotting using a **fusion protein** library of truncated preS2 fragments of different lengths, mAbs were assigned to two groups which coincided with groups I and III previously described. All mAbs recognized linear epitopes and were glycosylation independent. Six out of eight fine-mapped mAbs recognized common epitopes located in the N-terminal part of the **preS** sequence between amino acids 131 and 144 (group I), and inhibited binding of HBsAg to polymd. human serum albumin. Only two mAbs recognized a C-terminal **HBV**-genotype-specific epitope covering amino acid residues 162 to 168 (group III). These mAbs bound to the highly variable proteolysis-sensitive hinge of preS2. Although four out of six mAbs targeted to immunodominant region I require the full-length sequence 131-L[Q/L]DPRVRGLY[F/L]PAG-144, two mAbs recognize the shorter and slightly C-terminal-shifted sequences 133-DPRVRGLY[F/L]-141 or 135-PVRGLY[F/L]PAG-144. Together with previously identified preS2 epitopes 133-DPRVRGL-139, 137-RGLYFPA-143, and 132-QDPR-135, these data indicate diversity of the immune response against epitopes within the same immunodominant region. This diversity may be generated by a labile secondary structure. Sequence anal. suggests the transition from an .alpha.-helix to a loop structure at this site.

L12 ANSWER 9 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:451001 CAPLUS
DOCUMENT NUMBER: 122:283391
TITLE: Expression in E. coli of the chimeric genes in which the PreS coding region of the surface antigen of hepatitis B virus was fused to a glutathione S-transferase gene
AUTHOR(S): Liu, Hui; Li, Zaiping; Yu, Xianming

CORPORATE SOURCE: Shanghai Inst. Biochem., Academia Sinica, 200131, Peop. Rep. China
SOURCE: Shengwu Huaxue Yu Shengwu Wuli Xuebao (1994), 26(5), 513-18
CODEN: SHWPAU; ISSN: 0582-9879
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

TI Expression in E. coli of the chimeric genes in which the PreS coding region of the surface antigen of hepatitis B virus was fused to a glutathione S-transferase gene

SO Shengwu Huaxue Yu Shengwu Wuli Xuebao (1994), 26(5), 513-18
CODEN: SHWPAU; ISSN: 0582-9879

AU Liu, Hui; Li, Zaiping; Yu, Xianming

AB Fusion genes in which the coding regions of the intact or partially deleted **preS** region of the surface antigen (HBsAg) of hepatitis B virus (**HBV**) was fused to a glutathione S-transferase (GST) gene were constructed and expressed in E. coli. The yield of the **fusion proteins** declined rapidly as the length of the HBsAg **preS** segment increased. Moreover, the **preS** region of the **fusion proteins** degraded markedly, and the major cleavage sites were estd. to be around a.a. 75 of the preS1 region and a.a. 130 and a.a. 165 in the preS2 region. The research conducted with a proteinase-deficient strain revealed that the proteinases responsible for the proteolysis in the **preS** region existed in several E. coli strains and were not related to the two major protein degrdn. systems, Lon and htpR. The advantage of the GST fusion system was discussed.

L12 ANSWER 10 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:694374 CAPLUS
DOCUMENT NUMBER: 121:294374
TITLE: Integrated hepatitis B virus X and 3' truncated preS/S sequences derived from human hepatomas encode functionally active transactivators
AUTHOR(S): Schlueter, Volker; Meyer, Markus; Hofschneider, Peter H.; Koshy, Rajen; Caselmann, Wolfgang H.
CORPORATE SOURCE: Max-Planck-Inst. Biochem., Univ. Munich, Munich, 81366, Germany
SOURCE: Oncogene (1994), 9(11), 3335-44
CODEN: ONCNES; ISSN: 0950-9232
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Integrated hepatitis B virus X and 3' truncated preS/S sequences derived from human hepatomas encode functionally active transactivators

SO Oncogene (1994), 9(11), 3335-44
CODEN: ONCNES; ISSN: 0950-9232

AU Schlueter, Volker; Meyer, Markus; Hofschneider, Peter H.; Koshy, Rajen; Caselmann, Wolfgang H.

AB The hepatitis B virus (**HBV**) frequently integrates into hepatocellular genomic DNA during viral infection. Transcriptional transactivators encoded by integrated **HBV** X and 3' truncated **preS**/S sequences are known to stimulate gene expression from homologous and heterologous promoters. Here we demonstrate that 21 of 26 (81%) hepatocellular carcinoma tissues/cell lines contain coding sequences for at least one of the two known transactivators. Four integrated X and three **preS**/S transactivator sequences contained in five isolates

from three hepatoma primary tissues or cell lines were used as examples to provide functionality of the encoded transactivators. In one case, where both X and **preS**/S sequences were present, dissection of X and **preS**/S transactivator sequences showed independent functionality. The investigation of X- and **preS**/S-specific RNA and protein expression revealed the existence of carboxyterminally truncated viral-cellular **fusion proteins** that were able to stimulate gene expression from the c-fos proto-oncogene promoter five- to ten-fold. These results demonstrate that structurally intact **HBV** transactivator sequences are integrated in the majority of **HBV** -assocd. HCCs/hepatoma cell lines. In all tested examples integrated DNAs had retained functionality as transactivators. This data thereby support indirectly the hypothesis of a possible involvement of **HBV** transactivators in liver cell proliferation and hepatocarcinogenesis.

L12 ANSWER 11 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:18975 CAPLUS
DOCUMENT NUMBER: 114:18975
TITLE: Production and use of preS polypeptides of hepatitis B virus
INVENTOR(S): Acs, George; Christman, Judith K.; Price, Peter; Offensperger, Wolf; Wahl, Silke
PATENT ASSIGNEE(S): Mount Sinai School of Medicine, USA
SOURCE: U.S., 7 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 4959323	A	19900925	US 1985-794504	19851104

TI Production and use of preS polypeptides of hepatitis B virus
SO U.S., 7 pp.
CODEN: USXXAM
IN Acs, George; Christman, Judith K.; Price, Peter; Offensperger, Wolf; Wahl, Silke
AB **Fusion proteins** of hepatitis B virus (**HBV**) **preS** polypeptides and Escherichia coli .beta.-galactosidase are prepd. in a recombinant microorganism e.g. E. coli. The **fusion proteins** are useful in diagnosis of **HBV** infection and the purified **preS** polypeptides in immunization against **HBV**. Plasmid pWS3 encoding the preS2-.beta.-galactosidase **fusion protein** was constructed based on a high-expression vector pSKS105. Purifn. of the **fusion protein** and the preS2 polypeptide as well as their use as a diagnostic by ELISA were also described.

L12 ANSWER 12 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1986:86669 CAPLUS
DOCUMENT NUMBER: 104:86669
TITLE: Characterization of large surface proteins of hepatitis B virus by antibodies to preS-S encoded amino acids
AUTHOR(S): Pfaff, Eberhard; Klinkert, Mo Quen; Theilmann, Lorenz;

Schaller, Heinz
CORPORATE SOURCE: Dep. Microbiol., Univ. Heidelberg, Heidelberg, 6900, Fed. Rep. Ger.
SOURCE: Virology (1986), 148(1), 15-22
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Characterization of large surface proteins of hepatitis B virus by antibodies to preS-S encoded amino acids
SO Virology (1986), 148(1), 15-22
CODEN: VIRLAX; ISSN: 0042-6822
AU Pfaff, Eberhard; Klinkert, Mo Quen; Theilmann, Lorenz; Schaller, Heinz
AB The major surface protein of hepatitis B virus (**HBV**) the 226-amino acid hepatitis B surface antigen, is encoded in the 3'-proximal segment of the **preS**-S gene of 389 codons. To identify gene products from the 5' proximal **preS** sequence, DNA fragments from the **preS** region were expressed in Escherichia coli as **fusion proteins**. Antisera prepd. against these fusions were used to screen serum proteins of **HBV**-infected individuals, and found to react specifically with the 2 large **HBV** surface proteins of 39 and 42 kilodaltons. The presence of these proteins could be correlated with acute **HBV** infection. Anal. by Western blotting using the **preS** sequence-specific antisera and **HBV** particles sepd. into spheres, filaments, and Dane particles confirmed that these proteins were assocd. with the native virus. Dane particles contg. active DNA polymerase could be immune pptd. by the **preS**-specific antibodies, showing that the **preS**-coded part of these surface proteins is located on the surface of the virion.

L12 ANSWER 13 OF 18 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1999:349693 BIOSIS
DOCUMENT NUMBER: PREV199900349693
TITLE: Expression and characterization of chimeric hepatitis B surface antigen particles carrying preS epitopes.
AUTHOR(S): Hui, Jingyi; Li, Guangdi; Kong, Yuying; Wang, Yuan (1)
CORPORATE SOURCE: (1) Shanghai Institute of Biochemistry, Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai, 200031 China
SOURCE: Journal of Biotechnology, (June 11, 1999) Vol. 72, No. 1-2,
pp. 49-59.
ISSN: 0168-1656.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
TI Expression and characterization of chimeric hepatitis B surface antigen particles carrying preS epitopes.
SO Journal of Biotechnology, (June 11, 1999) Vol. 72, No. 1-2, pp. 49-59.
ISSN: 0168-1656.
AU Hui, Jingyi; Li, Guangdi; Kong, Yuying; Wang, Yuan (1)
AB Many studies have provided evidence that hepatitis B surface antigen (HBsAg) including preS1 and preS2 sequences could be an ideal candidate for a new hepatitis B virus (**HBV**) vaccine with higher efficacy. However, the large (L) protein containing the entire **preS** region expressed in mammalian cells is not efficiently assembled into particles and secreted. Here we report an alternative approach to include the dominant epitopes of preS1 and preS2 to the small (S) protein as **fusion proteins** by the recombinant DNA technology. Three **fusion proteins** containing preS2(120-146) and preS1(21-47) at the N-terminus and/or truncated C-terminus of S protein were expressed using the recombinant vaccinia virus system. All these

fusion proteins were efficiently secreted in the particulate form, and displayed S, preS1 and/or preS2 antigenicity. Further analysis showed that these chimeric HBsAg particles elicited strong antibody responses against S, preS1 and preS2 antigens in BALB/c mice, suggesting thatthey could be promising candidates for a new recombinant vaccine to induce broader antibody response required for protection against hepatitis B viral infection.

L12 ANSWER 14 OF 18 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1996:422905 BIOSIS
DOCUMENT NUMBER: PREV199699153961
TITLE: Visualization of hepatitis B virus (HBV) surface protein binding to HepG2 cells.
AUTHOR(S): Lee, Dong-Gun; Park, Jung-Hyun; Choi, Eun-A.; Han, Mi-Young; Kim, Kil-Lyong; Hahm, Kyung-Soo (1)
CORPORATE SOURCE: (1) Peptide Eng. Res. Unit, Korea Res. Inst. Bioscience and Biotechnology, KIST, Taejon 305-600 South Korea
SOURCE: Journal of Biochemistry and Molecular Biology, (1996) Vol. 29, No. 2, pp. 175-179.
ISSN: 1225-8687.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Visualization of hepatitis B virus (HBV) surface protein binding to HepG2 cells.
SO Journal of Biochemistry and Molecular Biology, (1996) Vol. 29, No. 2, pp. 175-179.
ISSN: 1225-8687.
AU Lee, Dong-Gun; Park, Jung-Hyun; Choi, Eun-A.; Han, Mi-Young; Kim, Kil-Lyong; Hahm, Kyung-Soo (1)
AB Viral surface proteins are known to play an essential role in attachment of the virus particle to the host cell membrane. In case of the hepatitis B virus (**HBV**) several reports have described potential receptors on the target cell side, but no definite receptor protein has been isolated yet. As for the viral side, it has been suggested that the **preS** region of the envelope protein, especially the preS1 region, is involved in binding of **HBV** to the host cell. In this study, preS1 region was recombinantly expressed in the form of a maltose binding protein (MBP) **fusion protein** and used to identify and visualize the expression of putative **HBV** receptor(s) on the host cell. Using laser scanned confocal microscopy and by FACS analysis, MBP-preS1 proteins were shown to bind to the human hepatoma cell line HepG2 in a receptor-ligand specific manner. The binding kinetic of MBP-preS1 to its cellular receptor was shown to be temperature and time dependent. In cells permeabilized with Triton X-100 and treated with the **fusion protein**, a specific staining of the nuclear membrane could be observed. To determine the precise location of the receptor binding site within the preS1 region, several short overlapping peptides from this region were synthesized and used in a competition assay. In this way the receptor binding epitope in preS1 was revealed to be amino acid residues 27 to 51, which is in agreement with previous reports. These results confirm the significance of the preS1 region in virus attachment in general, and suggest an internalization pathway mediated by direct attachment of the viral particle to the target cell membrane.

L12 ANSWER 15 OF 18 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:443427 BIOSIS
DOCUMENT NUMBER: PREV199598457727
TITLE: Gene fusion of cholera toxin B subunit and HBV PreS2

epitope and the antigenicity of **fusion protein**.
AUTHOR(S): Shi, Cheng-Hua (1); Cao, Cheng; Zhig, Jing-Sheng; Li, Jiezhi; Ma, Qing-Jun
CORPORATE SOURCE: (1) Mol. Genetics Cent., Inst. Biotechnol., Beijing 100850 China
SOURCE: Vaccine, (1995) Vol. 13, No. 10, pp. 933-937. ISSN: 0264-410X.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Gene fusion of cholera toxin B subunit and HBV PreS2 epitope and the antigenicity of **fusion protein**.
SO Vaccine, (1995) Vol. 13, No. 10, pp. 933-937. ISSN: 0264-410X.
AU Shi, Cheng-Hua (1); Cao, Cheng; Zhig, Jing-Sheng; Li, Jiezhi; Ma, Qing-Jun
AB A unique EcoRI site was introduced at the 3' end of cholera toxin B subunit (CTB) gene by site-directed mutagenesis, polynucleotides encoding 120-145aa epitope of **HBV PreS**-2 were chemically synthesized and fused to the 3' end of cholera toxin B subunit gene. The fused gene was over-expressed (about 30 mu-g ml-1) in E. coli, and more than 95% of the **fusion protein** was secreted into the medium. The **fusion protein** expressed was purified by affinity chromatography. The chimera protein obtained bound to ganglioside GM1, and had the antigenicity of both cholera toxin B subunit and **HBV** PreS2 as confirmed by ELISA. After mice were immunized intramuscularly with the **fusion protein**, anti-CTB antibody and anti-PreS2 antibody were produced. These results indicated that the **fusion protein** retained not only the biological function of CTB but also the antigenicity and the immunogenicity of cholera toxin B subunit and **HBV** PreS2 epitope. This work provided a sound basis for further studies on the construction of engineered peptide vaccine.

L12 ANSWER 16 OF 18 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:439804 BIOSIS
DOCUMENT NUMBER: PREV199598454104
TITLE: Fine mapping and functional characterization of two immuno-dominant regions from the preS2 sequence of hepatitis B virus.
AUTHOR(S): Meisel, Helga; Sominskaya, Irina; Pumpens, Pauls; Pushko, Peter; Borisova, Galina; Deepen, Ralf; Lu, Xuangyong; Spiller, Gerald H.; Krueger, Detlev H.; Grens, Elmars; Gerlich, Wolfram H. (1)
CORPORATE SOURCE: (1) Inst. Med. Virol., Justus-Liebig-Univ., Frankfurter Str. 107, D-35392 Giessen Germany
SOURCE: Intervirology, Vol. 37, No. 6, pp. 330-339. ISSN: 0300-5526.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Fine mapping and functional characterization of two immuno-dominant regions from the preS2 sequence of hepatitis B virus.
SO Intervirology, Vol. 37, No. 6, pp. 330-339. ISSN: 0300-5526.
AU Meisel, Helga; Sominskaya, Irina; Pumpens, Pauls; Pushko, Peter; Borisova, Galina; Deepen, Ralf; Lu, Xuangyong; Spiller, Gerald H.; Krueger, Detlev H.; Grens, Elmars; Gerlich, Wolfram H. (1)
AB A set of monoclonal antibodies (mAbs) directed against the preS2 region of

hepatitis B virus (**HBV**) surface antigen (HBsAg) was generated by immunization of mice with native HBsAg isolated from the blood of **HBV** carriers. According to (1) mutual competition binding of mAb to natural HBsAg, (2) recognition of full-length preS2 displayed on hepatitis B core particles, (3) recognition of synthetic partial preS2 peptides, and (4) Western blotting using a **fusion protein** library of truncated preS2 fragments of different lengths, mAbs were assigned to two groups which coincided with groups I and III described by Mimms et al. (Virology 1990; 176:604-6191. All mAbs recognized linear epitopes and were glycosylation independent. Six out of eight fine-mapped mAbs recognized common epitopes located in the amino-terminal part of the **preS** sequence between amino acids 131 and 144 (group 1), and inhibited binding of HBsAg to polymerized human serum albumin. Only two mAbs recognized a carboxy-terminal **HBV** -genotype-specific epitope covering amino acid residues 162 to 168 (group III). These mAbs bound to the highly variable proteolysis-sensitive hinge of preS2. Although four out of six mAbs targeted to immunodominant region I require the full-length sequence 131-L(Q/L)DPRVRGLY(F/L)PAG-144, two mAbs recognize the shorter and slightly carboxy-terminal-shifted sequences 133-DPRVRGLY(F/L)-141 or 135-PVRGLY(F/L)PAG-144. Together with previously identified preS2 epitopes 133-DPRVRGL-139, 137-RGLYFPA-143, and 132-QDPR-135, these data indicate diversity of the immune response against epitopes within the secondary structure. Sequence analysis suggests the transition from an a-helix to a loop structure at this site.

L12 ANSWER 17 OF 18 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:548249 BIOSIS
DOCUMENT NUMBER: PREV199598007797
TITLE: Integrated hepatitis B virus X and 3' truncated preS/S sequences derived from human hepatomas encode functionally active transactivators.
AUTHOR(S): Schlueter, Volker (1); Meyer, Markus; Hofschneider, Peter H.; Koshy, Rajen; Caselmann, Wolfgang H.
CORPORATE SOURCE: (1) Max-Planck-Inst. Biochemie, Dep. Virus Research, 82152 Martinsried Germany
SOURCE: Oncogene, (1994) Vol. 9, No. 11, pp. 3335-3344. ISSN: 0950-9232.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Integrated hepatitis B virus X and 3' truncated preS/S sequences derived from human hepatomas encode functionally active transactivators.
SO Oncogene, (1994) Vol. 9, No. 11, pp. 3335-3344. ISSN: 0950-9232.
AU Schlueter, Volker (1); Meyer, Markus; Hofschneider, Peter H.; Koshy, Rajen; Caselmann, Wolfgang H.
AB The hepatitis B virus (**HBV**) frequently integrates into hepatocellular genomic DNA during viral infection. Transcriptional transactivators encoded by integrated **HBV** X and 3' truncated **preS**/S sequences are known to stimulate gene expression from homologous and heterologous promoters. Here we demonstrate that 21 of 26 (81%) hepatocellular carcinoma tissues/cell lines contain coding sequences for at least one of the two known transactivators. Four integrated X and three **preS**/S transactivator sequences contained in five isolates from three hepatoma primary tissues or cell lines were used as examples to prove functionality of the encoded transactivators. In one case, where both X and **preS**/S sequences were present, dissection of X and

preS/S transactivator sequences showed independent functionality. The investigation of X- and **preS**/S-specific RNA and protein expression revealed the existence of carboxyterminally truncated viral-cellular **fusion proteins** that were able to stimulate gene expression from the c-fos proto-oncogene promoter five- to ten-fold. These results demonstrate that structurally intact **HBV** transactivator sequences are integrated in the majority of **HBV** -associated HCCs/hepatoma cell lines. In all tested examples integrated DNAs had retained functionality as transactivators. This data thereby support indirectly the hypothesis of a possible involvement of **HBV** transactivators in liver cell proliferation and hepatocarcinogenesis.

L12 ANSWER 18 OF 18 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1986:177646 BIOSIS
DOCUMENT NUMBER: BA81:88062
TITLE: CHARACTERIZATION OF LARGE SURFACE PROTEINS OF HEPATITIS B VIRUS BY ANTIBODIES TO PRE-S-S ENCODED AMINO-ACIDS.
AUTHOR(S): PFAFF E; KLINKERT M-Q; THEILMANN L; SCHALLER H
CORPORATE SOURCE: DEP. MICROBIOL., UNIV. HEIDELBERG, IM NEUENHEIMER FELD 282,
6900 HEIDELBERG, W. GERMANY.
SOURCE: VIROLOGY, (1986) 148 (1), 15-22.
CODEN: VIRLAX. ISSN: 0042-6822.
FILE SEGMENT: BA; OLD
LANGUAGE: English
TI CHARACTERIZATION OF LARGE SURFACE PROTEINS OF HEPATITIS B VIRUS BY ANTIBODIES TO PRE-S-S ENCODED AMINO-ACIDS.
SO VIROLOGY, (1986) 148 (1), 15-22.
CODEN: VIRLAX. ISSN: 0042-6822.
AU PFAFF E; KLINKERT M-Q; THEILMANN L; SCHALLER H
AB The major surface protein of **HBV**, the 226-amino-acid HBsAg, is encoded in the 3' proximal segment of the **preS**-S gene of 389 codons. To identify gene products from the 5' proximal **preS** sequence, DNA fragments from the **preS** region were expressed in Escherichia coli as **fusion proteins**. Antisera prepared against these fusions were used to screen serum proteins of **HBV** -infected individuals, and found to react specifically with the two large **HBV** surface proteins of 39 and 42 kDa. The presence of these proteins could be correlated with acute **HBV** infection. Analysis by Western blotting using the **preS** sequence-specific antisera and **HBV** particles separated into spheres, filaments, and Dane particles confirmed that these proteins were associated with the native virus. Dane particles containing active DNA polymerase could be immune precipitated by the **preS**-specific antibodies, showing that the **preS**-coded part of these surface proteins is located on the surface of the virion.